Journal of Clinical Immunology (2019) 39:823–826
https://doi.org/10.1007/s10875-019-00682-9

LETTER TO EDITOR

STK4 Deficiency in a Patient with Immune Complex Glomerulonephritis, Salt-Losing Tubulopathy, and Castleman's-Like Disease

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Received: 4 April 2019 / Accepted: 16 August 2019 / Published online: 23 August 2019

Keywords Primary immunodeficiency · combined immunodeficiency diseases · molecular genetics · autosomal recessive · infection · renal · Gitelman syndrome · NGS · MST1

To the Editor:

Combined immunodeficiency diseases (CID) are a heterogeneous group of disorders which all share profound T cell dysfunction. The presence of autologous T cells complicates diagnosis as these exhibit varying degrees of functionality, allowing the patient to survive after the age of 2 years without immunologic reconstitution [1]. Patients with CID suffer from recurrent infections as well as a diverse array of clinical presentations which may include lymphoproliferation, immune dysregulation, malignancy, allergy, and organ-specific autoimmunity and/or autoimmune cytopenia [2]. In 2012, serine-threonine protein kinase 4 (*STK4*) gene deficiency was described by three independent groups as causing autosomal recessive CID characterized by profound CD4 lymphopenia together with recurrent bacterial and viral infections and mucocutaneous candidiasis [3–5]. Immune dysregulation and autoimmune manifestation were also noted. *STK4* codes for the ubiquitously expressed mammalian sterile 20-like kinase (MST1), a cytoplasmic protein which demonstrates both proapoptotic and antiapoptotic functions and is known to promote Fas-mediated apoptosis [3–5], as well as inhibiting autoimmunity through modulation of Foxo1 and Foxo3 (Foxo1/3) stability [6].

Here we report a novel stopgain mutation in *STK4* in a patient presenting with CID and autoimmune features. In addition, there was an unexpected renal component in the form of a salt-losing tubulopathy suggestive of an acquired Gitelman syndrome, immune complex glomerulonephritis, and Castleman's-like disease, thereby expanding the phenotypic spectrum associated with loss of this protein.

The patient is a 15-year-old female. She was the only child conceived by in vitro fertilization (IVF) pregnancy after 11 years of infertility, to consanguineous parents from Saudi Arabia. Mother suffered from diabetes and systemic lupus erythematosus (SLE) and the pregnancy reached full term. After birth, the patient developed respiratory distress syndrome (RDS) requiring mechanical ventilation and intensive care admission. An atrial septal defect (ASD) was detected after birth which resolved with no surgical intervention. The

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10875-019-00682-9) contains supplementary material, which is available to authorized users.

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child subsequently presented at the age of 4 years with a sudden onset of jaundice and found to have low hemoglobin, high reticulocyte count, high bilirubin, and a positive Coombs test (Table S1). She also had positive Donath-Landsteiner antibody and diagnosed to have cold autoimmune hemolytic anemia (AHA). She was treated with 1 g/kg of intravenous immunoglobulin (IVIG) and methylprednisolone. The patient went on to require multiple blood transfusions and underwent four consecutive plasmapheresis sessions and azathioprine with no improvements. Anti-CD20 (Rituximab) infusion was tried but the patient did not tolerate and so was discontinued. As therapeutic trial with vincristine infusions was given for 4 weeks and her AHA was still refractory and eventually after 5 months of presentation, she underwent splenectomy which stabilized her hemoglobin. She was kept on long-term penicillin V as prophylaxis.

During this admission at the age of 4 years and 5 months, she was also found to have hypokalemia and hypomagnesemia, and the urine dipstick was negative for hematuria or proteinuria (Table S2). Her hypokalemia and hypomagnesemia was attributed to a Gitelman syndrome-type renal tubulopathy and was treated with potassium and magnesium replacement. No genetic screening for any salt-losing tubulopathies was performed at this stage.

Beginning at the age of 7 years old, she started developing recurrent infections although she was off immunosuppressive medications. This included one-time *Klebsiella pneumoniae* sepsis, recurrent skin infections due to human papillomavirus, and herpes zoster on two different occasions. She also had recurrent supportive lymphadenitis (lymph node biopsy showed negative EBV), urinary tract infections, cellulitis with positive *Staphylococcus aureus*, and otitis media. As a result, the patient underwent an immunology work-up (Table S1). Her results showed leukocytosis with normal neutrophils and lymphocytes but high eosinophils. She also had hypergammaglobulinemia with normal antibodies titer to tetanus and pneumococcal and poor T cells response to mitogen. The patient had normal oxidative burst assay by flow cytometry. All were in a picture of immune dysregulation with T cell dysfunction. The patient responded well to prophylaxis antibiotic and anti-viral. The patient is also known to have bronchial asthma but no other atopic diseases.

She first developed low-grade proteinuria aged 6 which progressively got worse. She subsequently developed dipstick hematuria aged 12 which was monitored serially. At the age of 14, she was referred for evaluation to nephrologists as she had developed renal dysfunction in conjunction with nephrotic range proteinuria. Physical examination revealed generalized lymphadenopathy which was found to be metabolically active in positron emission tomography–computed tomography imaging scan. An autoimmune work-up was negative; this included screening for anti-nuclear antibody, anti-neutrophil cytoplasmic antibodies, and anti-Sjogren's syndrome A/B antibodies; she also had normal complement levels and her serology was negative for HIV and hepatitis B and C virus. Renal biopsy showed moderate to marked mesangial matrix expansion and hypercellularity associated with normal thickness of capillary walls and focal endocapillary proliferation with mesangial, subendothelial, intramembranous, and subepithelial electron-dense deposits. In addition, immunofluorescence revealed positive staining for IgA, IgM, C3, C1q, and Kappa and Lambda light chains indicating a chronic immune complex-mediated glomerulonephritis (Fig. 1a–d). She also underwent axillary lymph node excision biopsy which revealed morphological features that were suggestive of a lymphoproliferative disorder with Castleman's-like changes (Fig. 1e); however, it was negative for malignancy and the biopsy was also negative for EBV and HHV-8 virus, plus the stains for acid-fast bacilli and fungal were all negative. In view of a complication of immune complex nephritis and lymphoproliferative disorder suggestive of Castleman's disease, she was treated with 4 doses of rituximab (Fig. 1f).

Following recruitment under an IRB-approved informed consent, DNA from patient and parents was extracted from whole blood and genome-wide genotyping was conducted (refer to Supplementary Notes for Materials & Methods). A list of the patient's regions of homozygosity (ROH) is provided in Table S3 and is graphically shown in Fig. 1h. The sample was then assessed using targeted next-generation sequencing, an updated gene panel that is specific for coding and splice site regions of 162 known PID genes [7]. After combing through the data with our filtering pipeline (Fig. 1i), the only genic or splice site variant that survived was a stopgain mutation in *STK4* (NM_006282.4:c.1024C>T:p.R342*) which segregated with the disease state (Fig. 1g).

To assess whether this mutation caused the STK4 transcript to undergo nonsense-mediated RNA decay, we extracted RNA from patient lymphoblastoid cells and performed real-time RT-PCR. Data revealed a significant drop of > 60% in *STK4* expression levels in the patient versus healthy controls ($p = 0.0033$, Fig. 1j).

To determine how the mutation was affecting protein expression and stability, we probed an immunoblot with an anti-STK4/MST1 antibody. The data did not reveal any full-sized STK4 protein in the patient's lymphoblastoid cells (Fig. 1k) nor was any truncated STK4 observed corresponding to the calculated size of the mutant protein. In addition, levels of FoxO3a, a transcriptional activator which is a direct downstream target of STK4, showed a drastic loss of expression in patient cells in line with data observed for other STK4-deficient patients [3] and which was significant versus a panel of healthy controls (Fig. 1k).

Finally, to assess if our patient had an inherited form of Gitelman syndrome caused by a mutation in an established gene, we Sanger sequenced all exons and splice site regions of *SLC12A3*, *CLCNKA*, and *CLCNKB*. Although the latter

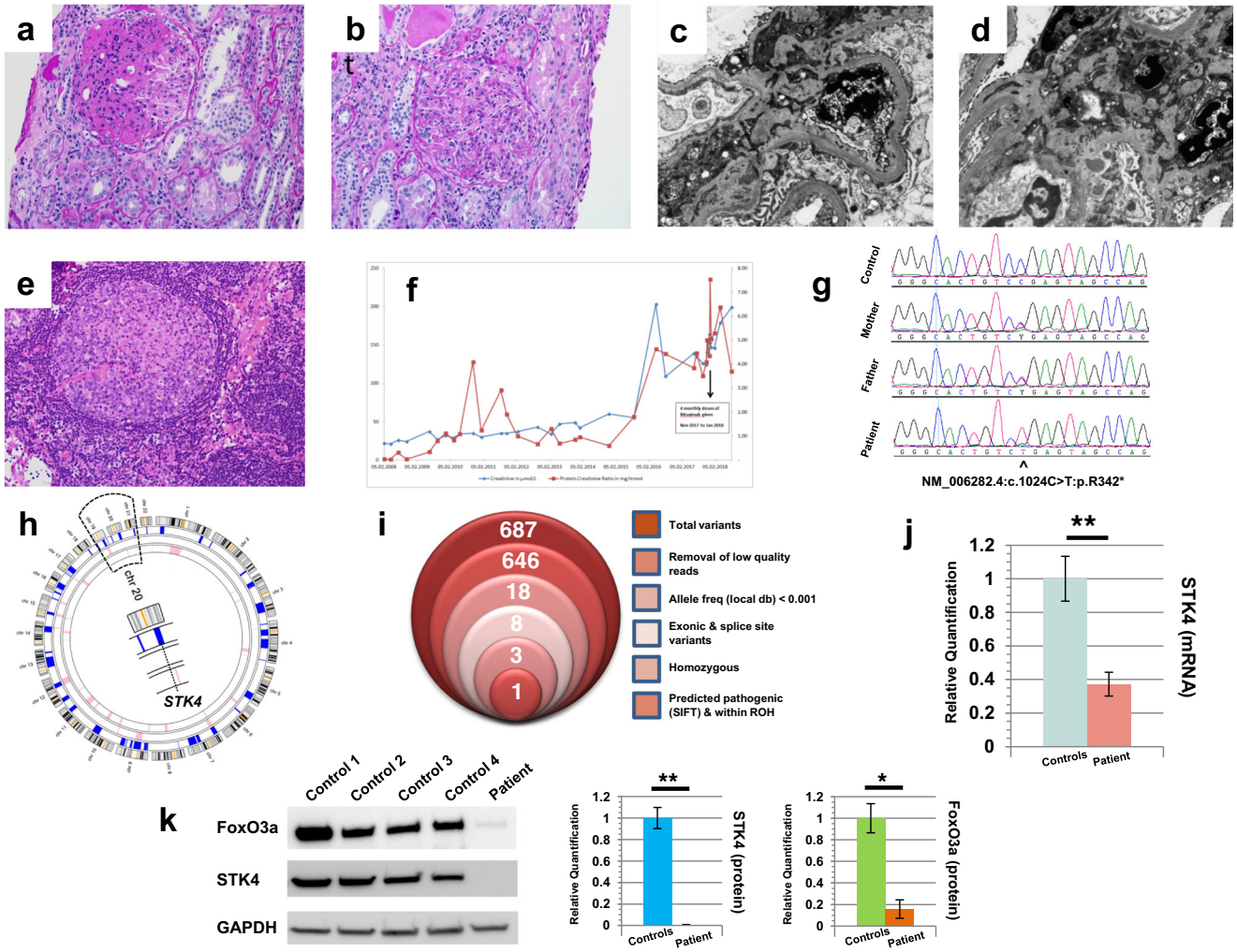

Fig. 1 A novel *STK4* mutation in a CID patient with autoimmunity. **a** Representative image of renal biopsy, which revealed eight glomeruli, one revealed global sclerosis, and three revealed segmental sclerosis. **b** Remaining glomeruli showed moderate to marked mesangial matrix expansion and hypercellularity associated with normal thickness of capillary walls and focal endocapillary proliferation. There was moderate interstitial fibrosis and tubular atrophy (30% of the cortical tissue), associated with moderate interstitial mononuclear inflammatory cell infiltrate admixed with plasma cells and few eosinophils, associated with foci of tubulitis. Immunofluorescence revealed positive staining for IgA, IgM, C3, C1q, and Kappa and Lambda light chains. **c** and **d** Ultrastructural examination demonstrated few scattered mesangial, subendothelial, intramembranous, and subepithelial electron dense deposits. The podocytes showed focal foot process effacement. **e** Left axillary lymph node biopsy, with morphological features suggestive of Castleman-like changes. **f** Creatinine and urine proteinuria over time (creatinine in μmol/L and urine protein creatinine ratio in mg/mmol). Urine protein creatinine ratio of 1 is equal to 1 g proteinuria over 24 h. Arrow and boxed text indicate when rituximab was administered. **g** Sequence chromatogram of the mutation and its segregation in the family, with control sequence given for reference. **h** Circular representation of chromosomes indicating regions of homozygosity (ROH) in the patient (dark blue) and the parents (pink). The *STK4* locus is indicated in the inset. **i** Stacked Venn diagram illustrating the total number of variants observed using targeted NGS, following inclusion of the indicated filters. **j** Real-time RT-PCR data of *STK4* expression levels in lymphoblastoid cells from our patient versus combined readings from three unrelated normal controls. Data were acquired through two independent experiments conducted in triplicate. **k** Left panel: immunoblot reveals absence of detectable STK4/MST1 protein in patient lymphoblastoid cells (no bands were detected at the calculated molecular weight of the truncated protein, data not shown). FoxO3a, a downstream target of STK4, is also significantly reduced in the patient. GAPDH serves as a loading control. Image is representative of three independent immunoblots. Right panels: relative quantification of STK4 and FoxO3a protein levels based on ImageJ analysis. Asterisks indicate significance levels (*$p < 0.05$, **$p < 0.01$; unpaired Student's *t* test). Error bars indicate SEMs

two are linked to Bartter syndrome, a recent publication highlighted a Gitelman patient with a possible digenic inheritance of *SLC12A3* and *CLCNKB* heterozygous mutations [8]. We therefore catalogued variants from all three genes in our patient (see Table S4). None of the genes harbored any variants which could be clearly pointed to as pathogenic.

We report here the first STK4 patient from the Arabian Peninsula, along with a novel stopgain mutation. The clinical and immunological presentation for CID due to STK4 mutation is variable and our patient had some of the common features reported previously such as recurrent viral skin infection, autoimmune cytopenia, congenital heart diseases, and

hypergammaglobulinemia [3, 5]. However, she did not have other features such as lymphopenia, intermittent neutropenia, lymphoma, or EBV lymphoproliferative disorder [9]. She had high eosinophilia but normal IgE and clinically, she had bronchial asthma and no other atopic diseases. Previously, Halacli et al. reported two patients mimicking autosomal recessive hyper IgE syndrome due to DOCK8 mutation [9], a finding that has been echoed recently by Moran et al. [10].

Our patient also had two additional findings, the first was a salt-losing tubulopathy associated with hypomagnesemia and hypokalemia and a chronic immune complex-mediated nephropathy with podocytopathy explaining the gradual loss of kidney function. There have been reports of acquired Gitelman syndrome patients with autoimmune diseases, such as the development of antibodies to thiazide-sensitive NaCl cotransporter (NCCT) resulting in tubular dysfunction presenting with hypokalemic metabolic alkalosis, hypomagnesemia, and hypocalciuria [11]. The second renal finding was a progressive proteinuric chronic kidney disease due to chronic immune complex glomerulonephritis. In addition, the excision biopsy of her lymph nodes revealed a morphology like Castleman's disease, an uncommon lymphoproliferative disorder characterized by enlarged hyperplastic lymph nodes [12]. This disease has known variants associated with HIV infection and human herpesvirus (HHV)-8; however, in our case, the HHV-8 staining was negative in the lymph node biopsy.

Hematopoietic stem cell transplant was considered but not perused in this patient, as her recurrent infections were controlled using prophylaxis antibiotic and anti-viral. Her complex presentation, affecting multiple organ systems, required a multidisciplinary approach. The renal presentation is unique and has not been previously reported, and we recommend that this now be screened in other patients who have STK4 deficiency.

Acknowledgments We thank the family members for their generous participation. We acknowledge the Saudi Human Genome Project for infrastructure and informatics support relating to the NGS work. We are also grateful to Salma M Wakil, Dorota Monies, Farrukh Sheikh, Sawsan Abu Awwad, and the sequencing and genotyping core facilities at KFSH&RC, for their invaluable assistance.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interests.

References

1. Roifman CM, Somech R, Kavadas F, Pires L, Nahum A, Dalal I, et al. Defining combined immunodeficiency. J Allergy Clin Immunol. 2012;130(1):177–83.
2. Picard C, al-Herz W, Bousfiha A, Casanova JL, Chatila T, Conley ME, et al. Primary immunodeficiency diseases: an update on the classification from the International Union of Immunological Societies Expert Committee for Primary Immunodeficiency 2015. J Clin Immunol. 2015;35(8):696–726.
3. Abdollahpour H, Appaswamy G, Kotlarz D, Diestelhorst J, Beier R, Schaffer AA, et al. The phenotype of human STK4 deficiency. Blood. 2012;119(15):3450–7.
4. Crequer A, Picard C, Patin E, D'Amico A, Abhyankar A, Munzer M, et al. Inherited MST1 deficiency underlies susceptibility to EV-HPV infections. PLoS One. 2012;7(8):e44010.
5. Nehme NT, Schmid JP, Debeurme F, Andre-Schmutz I, Lim A, Nitschke P, et al. MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. Blood. 2012;119(15):3458–68.
6. Du X, et al. Mst1/Mst2 regulate development and function of regulatory T cells through modulation of Foxo1/Foxo3 stability in autoimmune disease. J Immunol. 2014;192(4):1525–35.
7. Al-Mousa H, et al. Unbiased targeted next-generation sequencing molecular approach for primary immunodeficiency diseases. J Allergy Clin Immunol. 2016;137(6):1780–7.
8. Kong Y, Xu K, Yuan K, Zhu J, Gu W, Liang L, et al. Digenetic inheritance of SLC12A3 and CLCNKB genes in a Chinese girl with Gitelman syndrome. BMC Pediatr. 2019;19(1):114.
9. Halacli SO, Ayvaz DC, Sun-Tan C, Erman B, Uz E, Yilmaz DY, et al. STK4 (MST1) deficiency in two siblings with autoimmune cytopenias: a novel mutation. Clin Immunol. 2015;161(2):316–23.
10. Moran I, Avery DT, Payne K, Lenthall H, Davies EG, Burns S, et al. B cell-intrinsic requirement for STK4 in humoral immunity in mice and human subjects. J Allergy Clin Immunol. 2019;143(6):2302–5.
11. Blanchard A, Bockenhauer D, Bolignano D, Calò LA, Cosyns E, Devuyst O, et al. Gitelman syndrome: consensus and guidance from a kidney disease: improving global outcomes (KDIGO) controversies conference. Kidney Int. 2017;91(1):24–33.
12. El-Osta HE, Kurzrock R. Castleman's disease: from basic mechanisms to molecular therapeutics. Oncologist. 2011;16(4):497–511.